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14. ABSTRACT
The PI's laboratory has relocated to the University of Texas Health Science Center, San Antonio, TX. This is a report of the work that was conducted at AMC Cancer Research Center, Denver CO between March 1 and June 30, 2005. We have submitted a report describing the work performed in YI of the project in Feb 2005. This 4 month (February 26th 2005 to June 30th 2005) report describes the research accomplishments primarily associated with the Task 1 as outlined in the approved statement of work. As mentioned in the YI report we terminated the experiment involving feeding antioxidant supplemented diet in May 2005 (as described in our YI report). At the termination of the study (16 weeks), serum, prostate and other organs were collected from all the animals. The efficacy of the combination of antioxidants was assessed by histological evaluation of the prostate and modulation of antioxidant enzymes. Data indicate that 90% of the animals in the control group developed high grade PIN while only 10% of the animals in the experimental group developed high grade PIN. In contrast 90% of the animals on the experimental diet developed low grade PIN. This suggests that a combination of antioxidants can reduce or delay the appearance of high grade PIN in Noble rats. Detailed studies to understand the mechanism of action of these antioxidants as proposed are currently in progress.

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Introduction:

Although prostate cancer is considered to be a disease of older men, a significant number of relatively young men exhibit the earliest signs of prostate cancer. This suggests that the disease is initiated early and remains latent until some factors trigger it to become malignant. This long latency of prostate cancer progression provides an opportunity for intervention to prevent the initial disease from becoming cancerous. Since treatment options for prostate cancer are very limited for initial stages of the disease and unavailable for metastatic disease, it is imperative that other means to control the disease be vigorously tested to reduce the number of prostate cancer-related deaths in the United States.

Oxidants produced as by-products of cellular metabolism have been implicated in the genesis of prostate cancer. Oxidative stress is caused by an imbalance of cellular endogenous oxidant and antioxidant levels. Laboratory studies using different model systems indicate that oxidative stress markers increase and antioxidant enzyme levels decrease during prostate cancer progression. Oxidative stress generated by dietary fat and androgens has been implicated in prostate cancer. Further epidemiological studies with a variety of antioxidants such as selenium, tocopherols, lycopene, β-carotene etc. have been found to be effective in lowering prostate cancer risk. Although these data suggest the importance of oxidative stress and antioxidants in prostate cancer, they are flawed in that they do not add to our understanding of the nature and amounts of antioxidants that are beneficial. This is extremely important since several classes of oxidants are produced and

a single antioxidant cannot quench all the different species of oxidants produced from cellular metabolism. Further, time is an extremely important factor for successful antioxidant prophylaxis. Taken together, the stage of prostate development and the kinds of antioxidants used would play a major role in determining the success of antioxidant prophylaxis. This proposal is a first step in beginning to understand whether antioxidants can prevent or delay the formation of PIN. Based on evidence presented in the literature, ***we hypothesize that a combination of antioxidants can prevent or delay the development of Prostatic Intraepithelial Neoplasia in a T/E_2 model of PCA by modulating the level of oxidative stress markers and endogenous antioxidant levels.*** To test our hypothesis we propose three specific aims.

1) Determine the ability of antioxidants to prevent or delay the development of Prostatic Intraepithelial Neoplasia (PIN) and relate it to changes in T/E_2 in the serum and AR.
2) Determine the levels of oxidative stress markers of DNA, protein and lipids following antioxidant supplementation.
3) Determine the levels and functional ability of endogenous antioxidant components following antioxidant supplementation.

There has been no change in the specific aims proposed.

Key Research Accomplishments:

We focused solely on completing task 1 during this period (March 1 2005 through June 2005) as proposed in the grant application:

Task 1: Determine the ability of antioxidants to prevent or delay the development of Prostatic Intraepithelial Neoplasia (PIN) (**months 1-18**). This involves (a) breed Noble rats; (b) start antioxidant supplementation; (c) initiate PIN formation with hormone treatment; (d) terminate feeding protocol for 16 and 32 weeks.

We focused on task 1 during this time (March through June 30th 2005) as proposed in the grant application.

As mentioned in the YI report we terminated the experiment involving feeding antioxidant supplemented diet in May 2005 (as described in our YI report). At the termination of the study (16 weeks), serum, prostate and other organs were collected from all the animals. The efficacy of the combination of antioxidants was assessed by histological evaluation of the prostate and modulation of antioxidant enzymes. Data indicate that 90% of the animals in the control group developed high grade PIN while only 10% of the animals in the experimental group developed high grade PIN. In contrast 90% of the animals on the experimental diet developed low grade PIN. This suggests that a combination of antioxidants can reduce or delay the appearance of high grade PIN in Noble rats (Please see below for experimental details).

Antioxidant supplementation was non-toxic: Body weight of the animals on antioxidant and control diet was measured twice a week. Figure 1 shows mean$\pm$ sd of body weight from animals on control diet, low and high dose antioxidant diet as a function of age in weeks. There was no significant difference in the body weight of the animals between various groups.

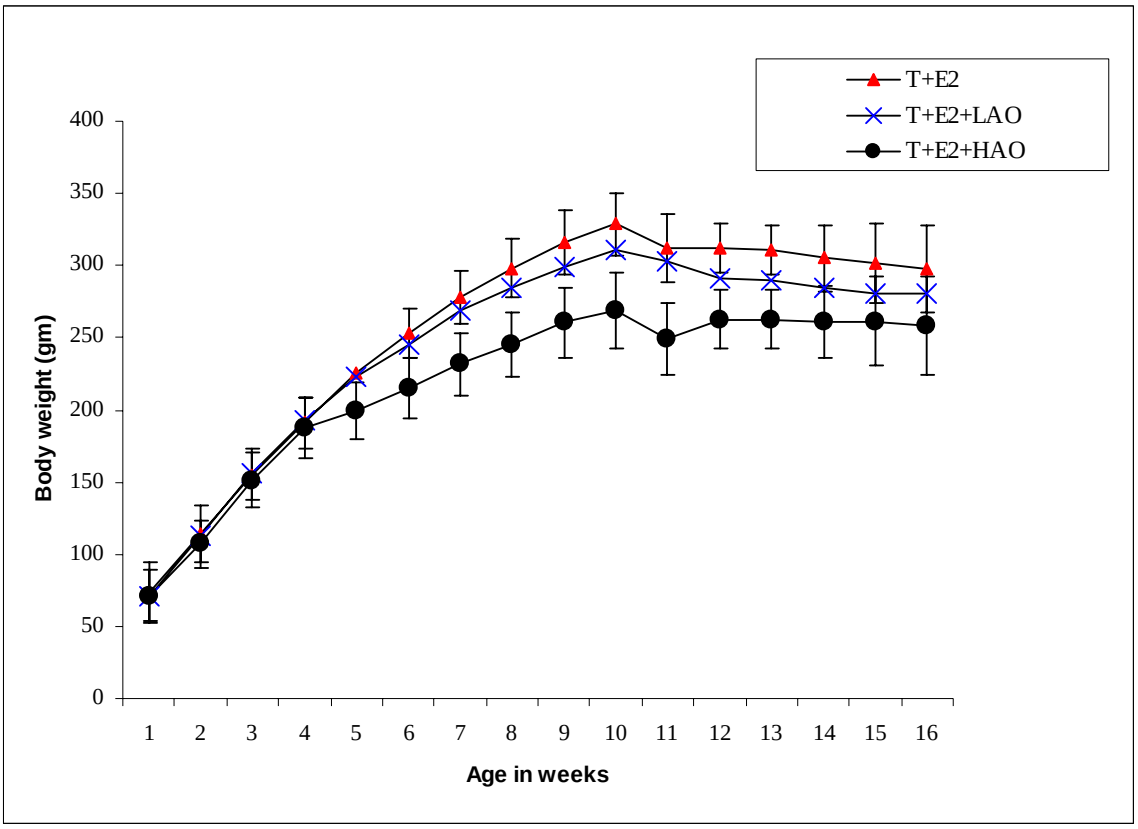
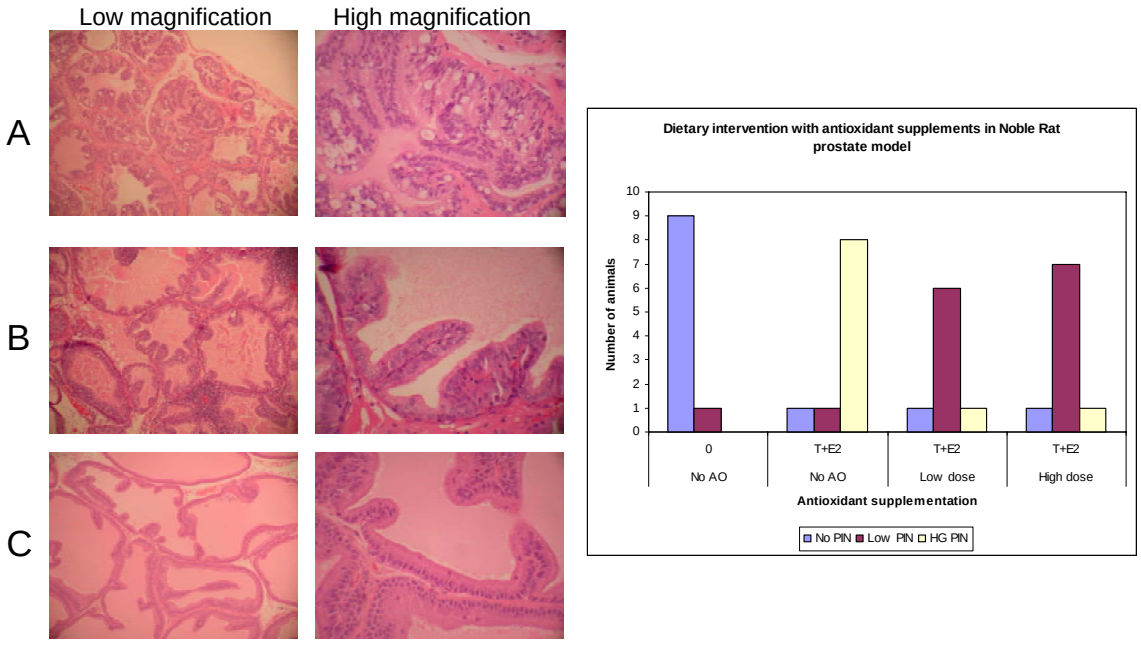


Figure 1

Effect of antioxidants on PIN: A portion of the prostate from these animals was fixed in formalin and evaluated for histological alterations using H&E analysis as well as scored for PIN. Data indicate that 90% of the animals in the control group developed high grade PIN while only 10% of the animals in the experimental group of animals developed high grade PIN. In contrast 90% of the animals on the experimental diet developed low grade PIN. As shown in Figure 2, prostate from animals on normal diet showed simple glands lined by single layer of cells with occasional papillary infoldings consistent with the published reports. Smaller but uniform nuclei without nucleoli can also be seen. On the other hand prostate from animals on normal diet but receiving hormones (testosterone and estradiol) displayed papillary infoldings, cells arranged in multiple layers, enlarged nuclei with nucleoli, clear vesicles along with decreased secretory material indicative of pre-malignant changes. On the other hand, we observed intermediate histological changes in the prostate from experimental group of animals with no significant difference in response to dose. However the dose H&E slides were shown in Figure 2. This suggests that combination of antioxidants can reduce or delay the appearance of high grade PIN in Noble rats.



Rat prostate showing simple glands with occasional papillary infoldings. The glands are lined by single layer of cells. The nuclei are smaller and more uniform with no nucleoli (Panel C); Prostate gland displaying pre-malignant histological changes showing cells with enlarged nuclei, nucleoli indicated by arrow, multiple cell layers with vacuolar changes, clear vesicles and decreased secretory material is seen in Panel A; Prostate gland with histological Changes intermediated between Panel A and C is shown in Panel B.

Figure 2

Detailed studies to understand the mechanism of action of these antioxidants as proposed are currently in progress.

As part of YII work we will continue making progress towards accomplishing the tasks as proposed in the original grant application at the University of Texas Health Science Center at San Antonio, TX. In aim 1, we will determine the levels of T and E2 in the serum, AR protein in the tumors to correlate with the observed histological changes. In aim 2, we will measure oxidative stress markers of DNA, proteins and lipids such as 8-oxo-dG, protein carbonyl content and lipid peroxidation products respectively will be assayed in the prostate tissue and serum obtained from control animals (with and without hormone stimulation) and animals on antioxidant supplemented diet.

Reportable outcomes: The outcome of the study is that antioxidant supplementation significantly reduced the development of high grade PIN. We have submitted an abstract to present our findings at the AACR meeting to be held in Washington D.C in 2006. We anticipate submitting a manuscript in the next 6-8 month period.

Conclusions: The major outcome from the study is antioxidant supplementation significantly reduced the development of high grade PIN. As proposed in the YI annual report during second year of grant period we anticipate demonstrating whether or not antioxidant prophylaxis can be used to prevent PIN formation. This will have a major impact in reducing prostate cancer incidence and mortality since it would nip the disease in the bud. We also expect to see a correlation between a particular oxidative stress marker and PIN inhibition it can be developed as a marker for predicting the disease risk. Since prostate cancer progresses asymptomatically

and slowly knowing the kinds and amounts of antioxidants and the stage of disease progression (pre or post neoplastic) when antioxidants offer the maximum protection could lead to a decrease in incidence or delay the development of clinically evident cancer. Please see the abstract submitted to AACR below.

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Prostate cancer (PCA) is the second leading cause of cancer related deaths in men. PCA progresses very slowly and occurs relatively late in life thus providing numerous opportunities for prevention of clinically significant disease. Although it is not clear whether appearance of prostatic intraepithelial neoplasia (PIN) predicts the appearance of prostate cancer in men, preneoplastic lesions have been found in young men and are fairly common in men in the fifties. However clinically detectable prostate cancer does not generally manifest until the age of 60 or 70. In addition, the occurrence of precancerous lesions is more prevalent (~1 in 3 men) than the incidence of carcinoma (~1 in 9 men; 3). It is of utmost importance at this juncture to develop strategies for the prevention of early stage prostate cancer to ensure quality of life for elderly men.

Noble rats develop prostate tumors in the dorso-lateral lobe of the prostate with biological characteristics similar to that of human PIN. Further this model develops prostate tumors in response to hormone stimulation making it an excellent preclinical model for targeting PIN. We used this model to test the ability of a mixture of antioxidants to prevent the development of PIN.

4-6 week old Noble rats were randomized into three groups. 20 animals in group I received normal diet; 10 animals in group II received a diet containing a low dose of a mixture of antioxidants while 10 animals in group III received a diet containing a low dose mixture of antioxidants. After 6 weeks group I animals were divided into sub-groups A and B. Group I B animals were implanted with Testosterone and Estradiol pellets while Group I A animals received placebo pellets. All animals in group II and III were implanted with Testosterone and Estradiol pellets. At the termination of the study (16 weeks), serum, prostate and other organs were collected from all the animals. The efficacy of the combination of antioxidants was assessed by histological evaluation of the prostate and modulation of antioxidant enzymes. Data indicate that 90% of the animals in the control group developed high grade PIN while only 10% of the animals in the experimental group developed high grade PIN. In contrast 90% of the animals on the experimental diet developed low grade PIN. This suggests that a combination of antioxidants can reduce or delay the appearance of high grade PIN in Noble rats. Supported by funds from the Department of Defense (W81 XWH-04-1-0275)